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Hybrid capture 2 viral load and the 2-year cumulative risk of cervical intraepithelial neoplasia grade 3 or cancer

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Objective: The purpose of this study was to determine the clinical value of a semiquantitative measure of human papillomavirus viral load by the hybrid capture 2 assay for stratification of the risk of histologic cervical intraepithelial neoplasia grade 3 or carcinoma.

Study design: The Atypical Cells of Unknown Significance and Low-Grade Squamous Intraepithelial Lesions Triage Study was a randomized clinical trial of 5060 women with 2 years of follow-up to evaluate treatment strategies for women with equivocal or mildly abnormal cervical cytologic condition. The usefulness of the continuous hybrid capture 2 output relative light units/positive controls that were above the positive threshold (1.0 relative light units/positive controls), which was a surrogate for human papillomavirus viral load, for distinguishing between hybrid capture 2 positive women who were diagnosed with cervical intraepithelial neoplasia grade 3 or carcinoma during the study from those who were not diagnosed with cervical intraepithelial neoplasia grade 3 or carcinoma was examined with the use of receiver-operator characteristic analyses.

Results: Relative light units/positive controls values did not further discriminate between hybrid capture 2 positive women with cervical intraepithelial neoplasia grade 3 or carcinoma from those with less than cervical intraepithelial neoplasia grade 3 or carcinoma. The use of a cervical intraepithelial neoplasia grade 2 or more severe or carcinoma case definition did not alter our findings.

Conclusion: Among women with atypical cells of unknown significance or low-grade squamous intraepithelial lesion cervical cytologic findings, the hybrid capture 2 viral load measurement did not improve the detection of 2-year cumulative cases of cervical intraepithelial neoplasia grade 3 or carcinoma significantly.

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Recognition that approximately 15 cancer-association (oncogenic) human papillomavirus (HPV) types cause virtually all cervical cancer worldwide[1-4] has led to the development of HPV DNA–targeted screening tests for the detection of cervical cancer and its immediate precursors. HPV DNA testing is now being introduced in the United States as an adjunct to cytologic screening and as a triage test to identify women with equivocal cytologic abnormalities (atypical squamous cells [ASCs]).[5,6] For screening, a single positive test for oncogenic HPV DNA predicts future increased risk for the development of cervical precancer (cervical intraepithelial neoplasia grade 3 [CIN3]) or cancer ($\geq$CIN3) and a negative test provides long-term reassurance.[7] As a triage test for women with equivocal cytologic findings, HPV DNA testing is very sensitive ($>$90%) for the detection of underlying $\geq$CIN3.[8,9] However, $>$50% of women with ASC are HPV DNA positive[10] and $<$10% of women with ASC have underlying histologic findings of $\geq$CIN3, which results in over-referral of patients.

Several studies now report that HPV16 viral load among women who test positive for HPV16 is predictive of incident cervical cancer and carcinoma in situ.[11-14] Thus, we were interested in exploring whether the semiquantitative viral load measurement from hybrid capture 2 (HC2; Digene Corporation, Gaithersburg, Md) can be used to predict the 2-year risk of high-grade cervical neoplasia (CIN2, CIN3, or cancer) among women who are infected with oncogenic HPV. A higher positive cut point (higher viral load) could increase clinical specificity and decrease sensitivity of this assay; this tradeoff must be assessed, given the importance of sensitivity in triage. Enrollment data from ALTS (Atypical Squamous Cells of Unknown Significance [ASCUS] and Low-Grade Squamous Intraepithelial Lesions [LSIL] Triage study),[15] a clinical trial to evaluate treatment strategies for women with either equivocal or mildly abnormal cervical cytologic findings that showed HC2 viral load does not significantly improve the detection of prevalent $\geq$CIN3.[16] Viral load values among women with CIN3 reflect the extent of surrounding histopathologic CIN1, the number of HPV types that are present, and the number of ASCUS/LSIL cells in exfoliative cervical samples, which contribute strongly to extreme variation in these values.[17] However, it has also been demonstrated that a single colposcopic evaluation with biopsy and histologic evaluation is not adequately sensitive for the detection of cervical precancer.[18] Recognizing that longitudinal ALTS data provide more complete ascertainment of prevalent disease and that in a 2-year follow-up period after a HPV DNA positive test, a $\geq$CIN3 diagnosis is more likely the result of missed prevalent disease than incident disease that develops from infection, we have reconsidered the clinical usefulness of HC2-measured viral load using ALTS cumulative $\geq$CIN3 and $\geq$CIN2.

Material and methods

Study design and population

ALTS was a randomized clinical trial that compared 3 treatment strategies for women with ASCUS or LSIL (Figure 1): immediate colposcopy, HPV triage, and conservative treatment; the latter was based on a program of repeat cytologic evaluation. Details of this study have been published.[10,15,18] Briefly, women with ASCUS or LSIL cytologic findings were recruited to participate in the study at 4 clinical centers: University of Alabama at Birmingham (Birmingham, Ala), Magee-Womens Hospital of the University of Pittsburgh Medical Center Health System (Pittsburgh, Pa), the University of Oklahoma (Oklahoma City, Okla), and the University of Washington (Seattle, Wash). The National Cancer Institute and local institutional review boards approved the study. A total of 5060 women enrolled in the study from January 1997 to December 1998: 3488 women with ASCUS and 1572 with LSIL cytologic findings. Routine follow-up and exit visits concluded in January 2001.

After eligibility was determined and written informed consent was obtained, participants were assigned randomly by referral stratum (ASCUS or LSIL) to 1 of the 3 treatment arms: immediate colposcopy (referral to colposcopy, regardless of enrollment test results), HPV triage (referral to colposcopy if the enrollment HPV result was positive or missing, or if the enrollment cytologic finding was high-grade squamous intraepithelial lesion), and conservative treatment (referral to colposcopy if the cytologic finding at enrollment or follow-up was high-grade squamous intraepithelial lesion). All women in each arm underwent the same enrollment pelvic examination, with the collection of 2 cervical specimens, the first in PreservCyt for ThinPrep cytologic evaluation (Cytyc Corporation, Boxborough, Mass) and HC2 testing and the second in specimen transport medium (Digene Corporation) for HPV DNA typing by polymerase chain reaction (PCR). Patient referral to colposcopy at enrollment was based on the randomization arm and enrollment test results. Women in all arms of the study and who were not lost to follow-up were re-evaluated by cytologic examination every 6 months for 2 years of follow-up. An exit examination, with colposcopy scheduled for all women, regardless of arm or previous procedures, was performed at 2 years of follow-up. We refer readers to other references for details on randomization, examination procedures, patient treatment, and laboratory and pathology methods.[10,15,18]

HPV testing

HC2 with the probe set B (henceforth, referred to as HC2) is a DNA test for 13 oncogenic HPV types. HC2

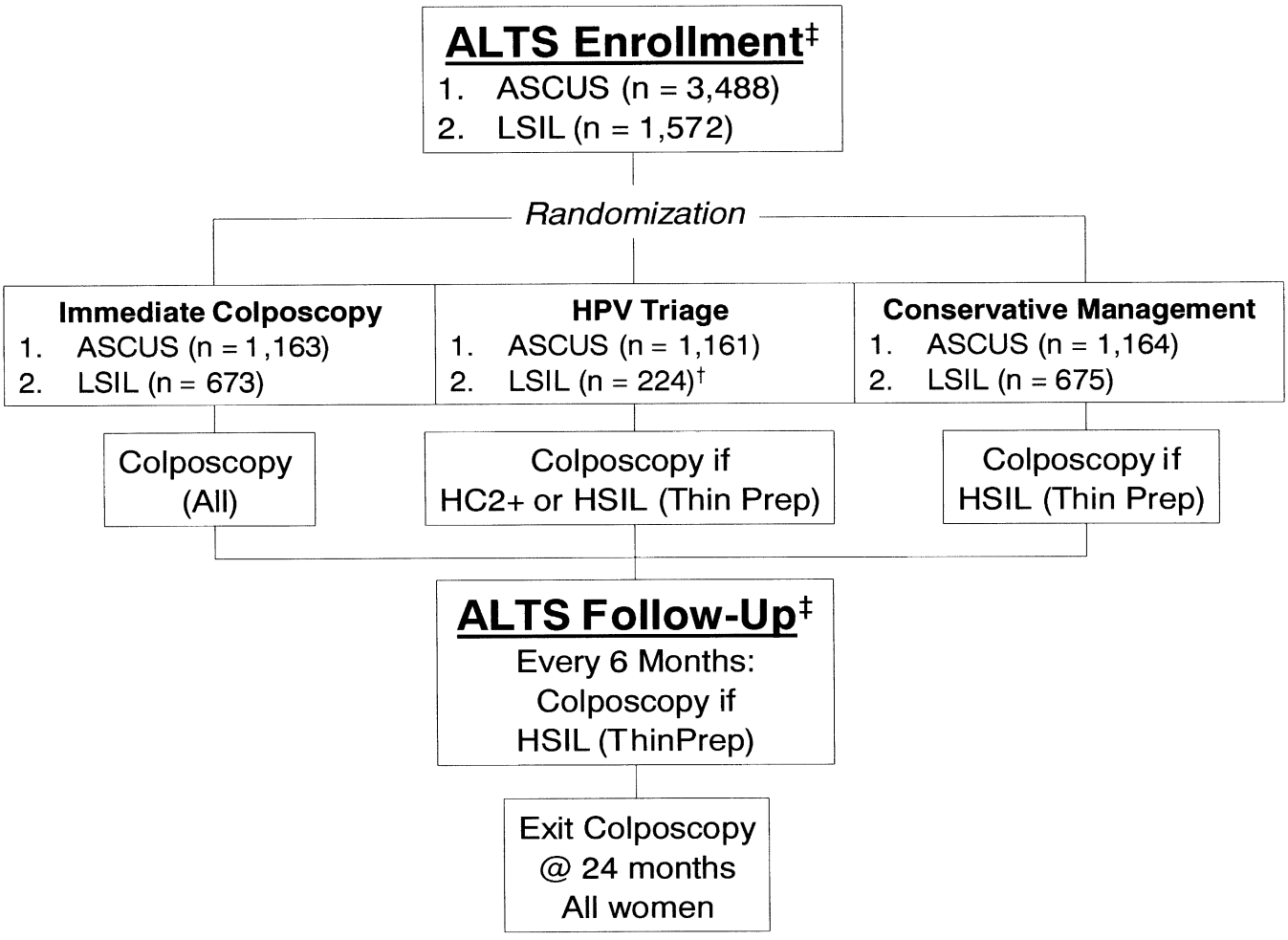

Figure 1 Flow diagram of ALTS trial. *Double dagger*, Suspected cancers (which were based on cervigram, cytologic, or histologic reviews) that were also referred to colposcopy at any time point; *dagger*, the HPV triage arm for women who were referred with a cytologic diagnosis of LSIL was closed early, because an interim analysis showed that 83% of these women would be triaged to colposcopy on the basis of a positive HPV result, which indicates a lack of clinical usefulness.[9]

relies on the formation of target HPV DNA-RNA probe heteroduplexes during the hybridization step in specimens positive for ≥1 oncogenic HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and the chemiluminescence detection of these hybrids by the use of an alkaline phosphatase-conjugated monoclonal antibody that is specific to DNA-RNA complexes with dioxetane substrate in a 96-well enzyme-linked immunosorbent assay format. After liquid-based ThinPrep cytology slides were prepared; aliquots of the residual in the PreservCyt vials were used for HPV DNA testing by HC2. Signal strengths in relative light units (RLUs) were compared with 1 pg/mL HPV type 16 DNA positive controls (RLU/PC). The Food and Drug Administration–approved 1.0 RLU/PC (approximately 1 pg/mL) was used as the threshold for a positive result.[15]

The continuous readout of HC2, RLU/PC has been shown to be a reasonable correlate of HPV viral load, as measured by real-time PCR.[19] Thus, RLU/PC values of >1.0 were used as a surrogate for viral load among women who tested positive for HC2. For the identification of women who were HC2 positive with a single type infection by HPV16, we used testing data that were based on L1 consensus primer PGMY09/11 PCR amplification and reverse-line blot hybridization for type-specific detection[20] on a the second cervical specimen that was collected into specimen transport medium.

Pathologic evaluation

Clinical treatment was based on the clinical center pathologists' cytologic and histologic diagnoses. In addition, all referral smears, ThinPreps, and histology slides were sent to the pathologic quality control group (QC pathology), which was based at Johns Hopkins Hospital for review and secondary diagnoses.

As the surrogate for cancer risk, we chose a priori a scientific end point of cumulative QC pathology–based histologic ≥CIN3 (n = 519 patients; note, only 7 cancers were diagnosed). However, for completeness, we

also examined case definitions of QC-diagnosed CIN2 (n = 381 patients), ≥CIN2 (n = 900 patients), and CIN3 (n = 512 patients) and the clinical end point of clinical center diagnosed CIN2 (n = 500 patients), ≥CIN2 (n = 895 patients), CIN3 (n = 388 patients), or ≥CIN3 (n = 395 patients).

Statistical analyses

As a point of reference, we calculated the sensitivity, specificity, positive predictive value, and negative predictive value for QC pathology diagnosed ≥CIN3 and for other QC and CC diagnosed case definitions. We included in our analyses those cases that were detected cumulatively, either at enrollment or during the 2-year follow-up. We used this rigorous definition of cases in recognition that (1) high-grade cervical neoplasia, especially ≥CIN3, that were detected within 2 years of an HPV-positive test is more likely to be a missed prevalent case than a true incident case because a single colposcopic evaluation with biopsy and histologic evaluation is not perfectly sensitive for the detection of cervical precancer and cancer[18] and (2) a good diagnostic test should detect the few early incident cases.

Receiver-operating characteristic (ROC) analyses[21] were used to evaluate the relationship of increasing RLU/PC values above the positive threshold of 1.0 to 2-year cumulative cases and to identify an inflection point that might be used to further discriminate among the HC2-positive women. A relative area under the ROC curve and corresponding 95% CI was calculated for HC2-positive women only. A relative area of 1 (100%) would indicate that a higher threshold (higher viral load) perfectly discriminates between cases and noncases (sensitivity, 100%; 1-specificity [nonspecificity], 0%). A relative area under the ROC curve of 0.5, demarcated by a diagonal, would indicate that increasing viral load does not further differentiate between cases and noncases (ie, random gains in sensitivity). A relative area under the ROC curve of <0.5 indicates worse than random selection of cases from noncases. ROC analyses were also performed on strata that were defined by referral cytologic finding into ALTS (ASCUS and LSIL) and age (<30 years old and ≥30 years old).

We examined the relationship of viral load and when ≥CIN3 was detected. Viral load above the 1.0 RLU/PC cut point was categorized into true quartiles, and time of detection was categorized as enrollment, early and late follow-up as defined by the median follow-up time, and exit. Stratified by study arm because of different enrollment procedures, standard contingency table methods with Pearson χ^2 tests and the Mantel-Haenszel extension test for trend were used to assess the possible associations of viral load and time to ≥CIN3 detection.

Results

As a point of reference, a summary of the clinical performance of HC2 test positivity for each case definition and for all women (ASCUS or LSIL) or women who were referred because of ASCUS cytologic finding is presented in the Table. Of all patients with valid HC2 test data (n = 4819/5060 patients; 95%), there were 3023 women (62.7%) who tested positive by HC2. With any of the case definitions, HC2 was >90% sensitive for 2-year cumulative cases of ≥CIN3.

The ROC curves to evaluate the relative performance above positive cut points for selected QC case definitions and strata are shown in the Figure 2 (the curve moves towards the origin as the RLU/PC value increases). The ROC analyses of viral load with the use of the case definitions of ≥CIN3 (Figure 2, *A*) demonstrate that higher viral load only marginally differentiates between HC2-positive cases and noncases (relative area under the ROC curve, 0.54; 95% CI, 0.52-0.57). The use of ≥CIN2 as the case definition (Figure 2, *B*; relative area under the ROC curve, 0.57; 95% CI, 0.54-0.59) or any definition of case that used the clinical center pathologists' cytologic diagnosis (data not shown) did not change this conclusion appreciably.

There was minimal value of viral load for the detection of QC pathology diagnosed ≥CIN3 in strata, defined by referral cytologic findings, ASCUS (relative area under the ROC curve, 0.56, 95% CI, 0.52-0.59; Figure 2, *C*) or LSIL (relative area under the ROC curve, 0.51; 95% CI, 0.48-0.55) or by age <30 years old (relative area under the ROC curve, 0.54; 95% CI, 0.51-0.56) or ≥30 years old (relative area under the ROC curve, 0.56; 95% CI, 0.50-0.63; Figure 2, *D*). Restricted to single type HPV 16 infections as determined by L1 consensus primer PGMY09/11 PCR amplification and reverse-line blot hybridization for type-specific detection, HC2 viral load did not distinguish between ≥CIN3 and <CIN3 (area under the ROC curve, 0.49; 95% CI, 0.41-0.57).

We then examined the time to detection of ≥CIN3, on the basis of viral load. Lower viral load was associated with the delayed detection of ≥CIN3 in all 3 arms of the study: immediate colposcopy (P_{Trend} = 0.06), HPV triage (P_{Trend} = 0.04), and conservative treatment (P_{Trend} = 0.006). Higher viral load was also associated with early CIN1 detection in all 3 study arms: immediate colposcopy (P_{Trend} = 0.001), HPV triage (P_{Trend} = 0.02), and conservative treatment (P_{Trend} = 0.003).

Comment

We demonstrated a lack of added clinical usefulness for the semiquantitative viral load measurement of HC2 RLU/PC in differentiating HC2-positive women with equivocal or mildly abnormal cytologic findings who

Table Summary of clinical performance of HC2 for QC and CC pathologic definitions of ≥CIN2 and ≥CIN3 for all ALTS participants and only those patients who were referred because of an ASCUS Papanicolaou test results

Group	Case definition	Cases (n)	Parameter	All cases (n = 4819)		ASCUS referral (n = 3326)	
				Value (%)	95% CI	Value (%)	95% CI
QC	≥CIN3	519	Sensitivity	93.6	91.2-95.6	92.4	88.8-95.2
			Specificity	41.0	39.5-42.5	50.6	48.8-52.4
			Positive predictive value	16.1	14.8-17.4	15.2	13.6-17.0
			Negative predictive value	98.2	97.4-98.7	98.6	97.9-99.1
	CIN3 only	512	Sensitivity	93.6	91.1-95.5	92.4	88.7-95.2
			Specificity	41.0	39.5-42.5	50.6	48.8-52.4
			Positive predictive value	15.9	14.6-17.2	15.1	13.5-16.9
			Negative predictive value	98.2	97.4-98.7	98.6	97.9-99.1
	≥CIN2	900	Sensitivity	93.7	91.9-95.2	92.0	89.3-94.2
			Specificity	44.4	42.8-45.9	54.0	52.1-55.8
			Positive predictive value	27.9	26.3-29.5	26.8	24.7-28.9
			Negative predictive value	96.8	95.9-97.6	97.4	96.4-98.1
	CIN2 only	381	Sensitivity	93.7	90.8-95.9	91.5	87.0-94.8
			Specificity	44.4	42.8-45.9	54.0	52.1-55.8
			Positive predictive value	14.1	12.7-15.5	13.6	11.9-15.5
			Negative predictive value	98.6	98.0-99.1	98.8	98.1-99.3
CC	≥CIN3	395	Sensitivity	95.9	93.5-97.7	96.2	93.0-98.3
			Specificity	40.2	38.8-41.7	50.2	48.4-52.0
			Positive predictive value	12.5	11.4-13.8	13.0	11.5-14.7
			Negative predictive value	99.1	98.6-99.5	99.4	98.9-99.7
	CIN3 only	388	Sensitivity	95.9	93.4-97.6	96.2	92.9-98.2
			Specificity	40.2	38.8-41.7	50.2	48.4-52.0
			Positive predictive value	12.3	11.2-13.6	12.9	11.3-14.5
			Negative predictive value	99.1	98.6-99.5	99.4	98.9-99.7
	≥CIN2	895	Sensitivity	92.6	90.7-94.3	91.0	88.1-93.3
			Specificity	44.1	42.5-45.7	53.7	51.8-55.6
			Positive predictive value	27.4	25.8-29.1	26.2	24.2-28.3
			Negative predictive value	96.3	95.3-97.1	97.0	96.1-97.8
	CIN2 only	500	Sensitivity	90.0	87.0-92.5	86.3	81.6-90.2
			Specificity	44.1	42.5-45.7	53.7	51.8-55.6
			Positive predictive value	17.0	15.6-18.5	15.2	13.4-17.1
			Negative predictive value	97.2	96.3-97.9	97.6	96.7-98.3

had underlying high-grade cervical neoplasia from those women who did not during the 2 years of ALTS. The relative areas under the ROC curves were trivially greater than 0.50, which suggests a near random tradeoff of sensitivity and specificity. More importantly, there were no inflection points that represented an obvious threshold for the selection of cases from noncases. We again verified that HC2 is a sensitive test for underlying cervical precancer, using a more rigorous criterion for detection: 2-year cumulative cases of ≥CIN3. However, HC2 is only modestly specific, so there would be benefit to further differentiate those women with and without high-grade disease. However, on the basis of these data, we argue against the use of the RLU/PC value, a semiquantitative measure of viral load, for decisions regarding clinical treatment.

The choice of 1.0 RLU/PC positive cut point was based on a population sample of disease.[22] In a group of women with equivocal or mildly abnormal cytologic findings, it was unclear whether this positive cut point choice was ideal. A recent study found that the use of 2.36 RLU/PC was the optimal cut point for triage of women with ASCUS cytologic findings for the detection of ≥CIN2.[23] ALTS provided the opportunity to re-examine the optimal HC2 cut point for triage of ASCUS, with a much larger sample size with greater numbers of true precancerous outcomes (CIN3) and extensive histopathologic review. An additional strength of this study was that it largely avoided verification bias (ie, differential case ascertainment among patients who are test positive vs those patients who are test negative, which fails to fully account for false-negative results) as consequences of our case definition (all enrollment, follow-up, and exit cases) and that 84% of the women had an exiting colposcopic evaluation and/or loop electrosurgical excision procedure.

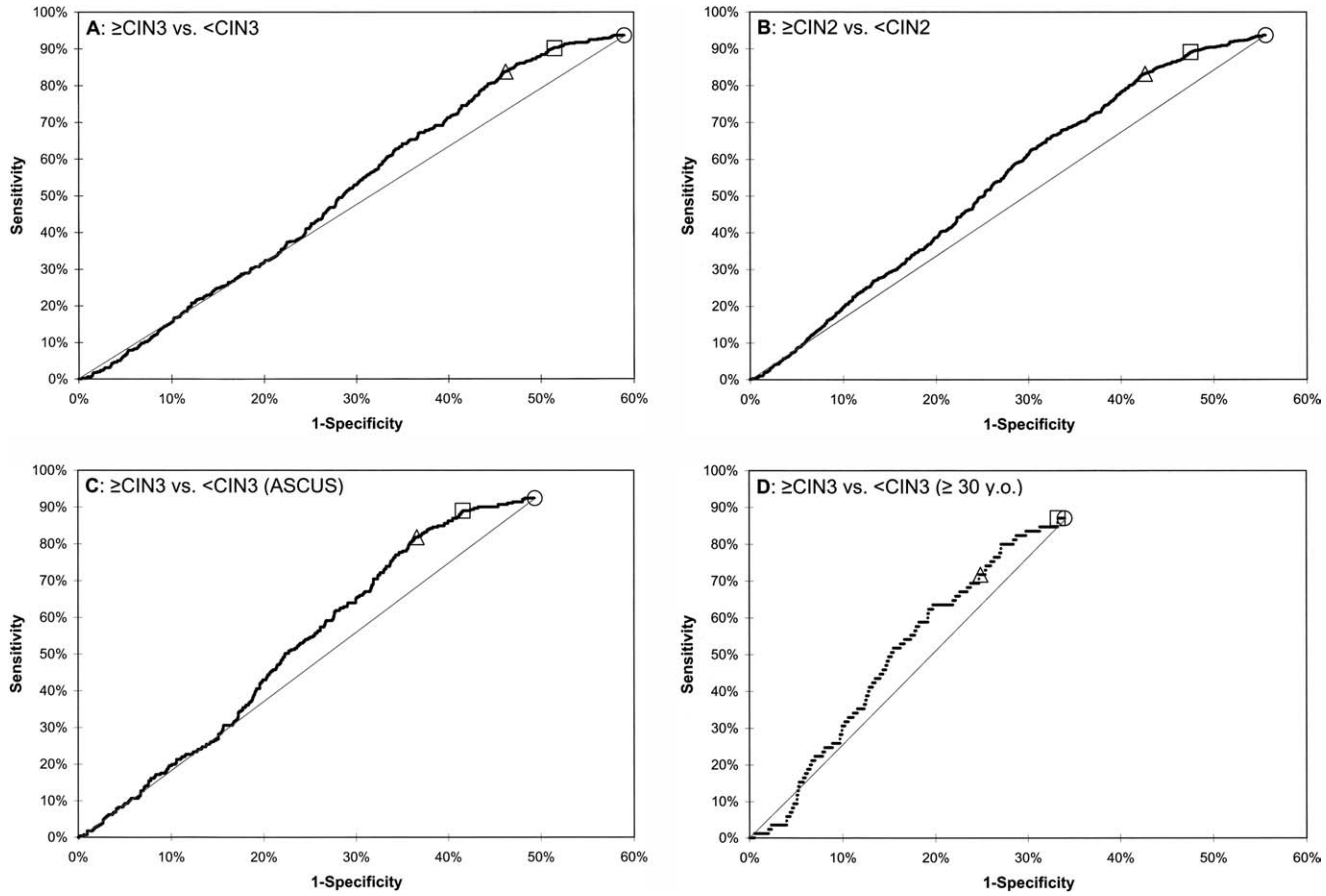

Figure 2 RLU/PC of the HC2 assay above the positive cut point of 1.0 RLU/PC for ≥CIN3 versus <CIN3 (**A**), ≥CIN2 versus <CIN2 (**B**), ≥CIN3 versus <CIN3 among ASCUS referrals (**C**), and ≥CIN3 versus <CIN3 among ≥30 year old women (**D**). Note that the 1.0 RLU/PC cut point is indicated by the *open circle*; reading the graph right to left, the absolute optimal threshold, which weights sensitivity and specificity equally, is indicated by the *open box*. As a second point of reference, a cut point of 10.0 RLU/PC is indicated by the *open triangle*. A relative area under the ROC curve is defined by the *data curve* for HC2-positive women, and the *gray diagonal line* indicates an ROC curve in which there was no improvement in performance that is attributable to the RLU/PC values of >1.0 (relative area, 0.5).

We note that the 1.0 RLU/PC would not be the optimal cut point for test accuracy (distinguishing ≥CIN3 from <CIN3), if sensitivity and specificity were weighted equally, in this group of higher risk women. For example, for women who were referred with ASCUS and with a ≥CIN3 case definition, a positive cut point of 3.76 RLU/PC would result in a slightly more accurate triage test (ie, a greater increase in specificity: 58.6% vs 50.6%) would compensate for the loss in sensitivity (88.7% vs 92.4%). It is likely that such a loss in sensitivity would not be considered acceptable for such a minor gain in specificity in a triage scenario for which sensitivity is critical. Comparatively, lowering the threshold to 0.7 RLU/PC would result in a 1.1% increase in sensitivity to 93.5% (which would result in the detection of 3 additional cases) and a 2.6% decrease in specificity to 48.0% (which would result in the referral of an additional 81 noncases to colposcopy).

Some recent reports have implicated viral load, particularly HPV16 viral load, as a risk factor for the development of invasive cancer.[11-14] Even in a subgroup of women who apparently were infected only with HPV16, high HC2 viral load did not distinguish between cases and noncases. We offer 3 explanations. First, we note that the cases that were evaluated in these analyses were primarily prevalent (ie, only those cases that occurred within a 2-year follow-up), whereas other studies may have included incident cases up to 26 years from baseline.

Second, although HC2 is a reasonable estimate of viral load,[19] real-time PCR may detect HPV DNA below the limits of detection by HC2, which uses positive cut points that are optimized not for the detection of HPV DNA but for the accurate discrimination of cervical precancer and cancer from lesser diagnoses.[22] With these "ultra" low viral load—positive women who may be at a very low risk of persistence and

progression compared with other infections as the referent group may elevate the relative risk of women with higher viral loads that are detectable by HC2. This population was selected for having cytologic abnormalities, which is synonymous with elevated viral loads; thus, we may have partially controlled for differences in viral load. Furthermore, HC2 does not provide typing; therefore, viral load may represent either the viral load of a single-type infection or the composite of the multiple-type infection. In this population, multiple-type infections were quite common, with 57% of HC2-positive women having >1 type of infection, as detected by PCR. Another limitation of HC2 is that it does not standardize for the cellularity of the specimen, which undoubtedly contributes to the variability of the measurement and therefore may lead to potentially greater misclassification of viral load status.

Finally, higher viral loads were strongly associated with CIN1 detection, which suggests that high viral loads in an intensely screened population could lead to treatment before the development of incident ≥CIN3. Indeed, in ALTS, women who had the highest viral load are more likely to undergo a loop electrosurgical excision procedure. This censoring of high viral load HPV infections may have obscured any observable association of viral load and early incident ≥CIN3.

It is also worth mentioning that there are other differences between this study and the aforementioned studies that found viral load to be predictive of cervical precancer and cancer: (1) those studies used different specimens from this study (eg, scraped cells from Papanicolaou test slides vs Papanicolaou specimens in PreservCyt) that were likely collected with different cervical samplers and (2) the cases were reviewed extensively in this study and not in the other studies. The impact of these differences is not obvious, but if the unreviewed cases were overcalled in the other studies, it can be anticipated that an association of higher viral load and cases would be observed because it might be expected that women with a high viral load at 1 time point might be more likely to have mild HPV-induced histopathologic findings in the future.

In summary, we found that HC2 is a sensitive triage test for the detection of cumulative 2-year ≥CIN3 in women with equivocal or mildly abnormal cytologic findings, which include some cases that are likely to be missed at enrollment colposcopy but to be detected within 2 years of follow-up. However, we also found that a single HC2 viral load surrogate measure was not useful clinically for further discriminating between HC2 test−positive women with ≥CIN3 from those HPV-infected women without ≥CIN3. Thus, HC2 positivity still results in the over referral of women with HPV infection but no cervical precancer. HC2 uses a probe set for the detection of 13 HPV types, and it is likely, as noted previously,[16,17,24] that viral load as measured by HC2 is misclassified because of multiple types. Whether serial measurements of viral load by HC2 might better distinguish between women with cervical precancer from those oncogenic HPV DNA−positive women without cervical precancer remains untested. It is possible that a type-specific clinical HPV test that also measures viral load at lower levels may be useful clinically, but this determination awaits the development and full validation of such a test.

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References

1. Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective: International biological study on cervical cancer (IBSCC) study group. J Natl Cancer Inst 1995;87:796-802.
2. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189:12-9.
3. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003; 348:518-27.
4. Wright TC Jr, Schiffman M. Adding a test for human papillomavirus DNA to cervical-cancer screening. N Engl J Med 2003; 348:489-90.
5. Saslow D, Runowicz CD, Solomon D, Moscicki AB, Smith RA, Eyre HJ, et al. American Cancer Society guideline for the early detection of cervical neoplasia and cancer. CA Cancer J Clin 2002;52:342-62.
6. Cuzick J, Szarewski A, Cubie H, Hulman G, Kitchener H, Luesley D, et al. Management of women who test positive for high-risk types of human papillomavirus: the HART study. Lancet 2003;362:1871-6.
7. Sherman ME, Lorincz AT, Scott DR, Wacholder S, Castle PE, Glass AG, et al. Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. J Natl Cancer Inst 2003;95:46-52.
8. Manos MM, Kinney WK, Hurley LB, Sherman ME, Shieh-Ngai J, Kurman RJ, et al. Identifying women with cervical neoplasia: using human papillomavirus DNA testing for equivocal Papanicolaou results. JAMA 1999;281:1605-10.
9. Solomon D, Schiffman M, Tarone R. Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial. J Natl Cancer Inst 2001;93:293-9.
10. ASCUS-LSIL Triage Study (ALTS) Group. Results of a randomized trial on the management of cytology interpretations of atypical squamous cells of undetermined significance. Am J Obstet Gynecol 2003;188:1383-92.
11. Dalstein V, Riethmuller D, Pretet JL, Le Bail CK, Sautiere JL, Carbillet JP, et al. Persistence and load of high-risk HPV are predictors for development of high-grade cervical lesions: a longitudinal French cohort study. Int J Cancer 2003;106:396-403.
12. Josefsson AM, Magnusson PK, Ylitalo N, Sorensen P, Qwarforth-Tubbin P, Andersen PK, et al. Viral load of human papilloma virus 16 as a determinant for development of cervical carcinoma in situ: a nested case-control study. Lancet 2000;355:2189-93.
13. Schlecht NF, Trevisan A, Duarte-Franco E, Rohan TE, Ferenczy A, Villa LL, et al. Viral load as a predictor of the risk of cervical intraepithelial neoplasia. Int J Cancer 2003; 103:519-24.
14. Ylitalo N, Sorensen P, Josefsson AM, Magnusson PK, Andersen PK, Ponten J, et al. Consistent high viral load of human papillomavirus 16 and risk of cervical carcinoma in situ: a nested case-control study. Lancet 2000;355:2194-8.
15. Schiffman M, Adrianza ME. ASCUS-LSIL triage study: design, methods and characteristics of trial participants. Acta Cytol 2000;44:726-42.
16. Sherman ME, Schiffman M, Cox JT. Effects of age and human papilloma viral load on colposcopy triage: data from the randomized Atypical Squamous Cells Of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesion Triage study (ALTS). J Natl Cancer Inst 2002;94:102-7.
17. Sherman ME, Wang SS, Wheeler CM, Rich L, Gravitt PE, Tarone R, et al. Determinants of human papillomavirus load among women with histological cervical intraepithelial neoplasia 3: dominant impact of surrounding low-grade lesions. Cancer Epidemiol Biomarkers Prev 2003;12:1038-44.
18. ASCUS-LSIL Triage Study (ALTS) group . A randomized trial on the management of low-grade squamous intraepithelial lesion cytology interpretations. Am J Obstet Gynecol 2003;188: 1393-400.
19. Gravitt PE, Burk RD, Lorincz A, Herrero R, Hildesheim A, Sherman ME, et al. A comparison between real-time polymerase chain reaction and hybrid capture 2 for human papillomavirus DNA quantitation. Cancer Epidemiol Biomarkers Prev 2003;12: 477-84.
20. Gravitt PE, Peyton CL, Alessi TQ, Wheeler CM, Coutlee F, Hildesheim A, et al. Improved amplification of genital human papillomaviruses. J Clin Microbiol 2000;38:357-61.
21. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 1993;39:561-77.
22. Schiffman M, Herrero R, Hildesheim A, Sherman ME, Bratti M, Wacholder S, et al. HPV DNA testing in cervical cancer screening: results from women in a high-risk province of Costa Rica. JAMA 2000;283:87-93.
23. Howard M, Sellors J, Kaczorowski J. Optimizing the hybrid capture II human papillomavirus test to detect cervical intraepithelial neoplasia. Obstet Gynecol 2002;100:972-80.
24. Sherman ME, Wang SS, Tarone R, Rich L, Schiffman M. Histopathologic extent of cervical intraepithelial neoplasia 3 lesions in the Atypical Squamous Cells of Undetermined Significance Low-grade Squamous Intraepithelial Lesion Triage study: implications for subject safety and lead-time bias. Cancer Epidemiol Biomarkers Prev 2003;12:372-9.